CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
BENSULIDE

Chemical Code # 000070, Tolerance # 00241
SB 950 # 022

Original date: 8/10/98; 6/28/99

Combined, rat:	No data gap, no adverse effect.
Chronic toxicity, rat:	See Combined, rat subchronic study.
Chronic toxicity, dog:	No data gap, no adverse effect.
Oncogenicity, rat:	See Combined rat.
Oncogenicity, mouse:	No data gap, no adverse effect.
Reproduction, rat:	No data gap, no adverse effect.
Teratology, rat:	No data gap, no adverse effect.
Teratology, rabbit:	No data gap, no adverse effect.
Gene mutation:	No data gap, no adverse effect.
Chromosome effects:	No data gap, no adverse effect.
DNA damage:	No data gap, no adverse effect.
Neurotoxicity:	No data gap, possible adverse effect indicated.

Toxicology one-liners are attached.

All record numbers through 165411 in 241-092 were examined.

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

File name: T990628

Toxicology summary: P. Iyer, 6/28/99

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

**079 150928 J. Willerton, C. Atkinson and M. Peterson-Jones., "Bensulide: 104 Week Dietary Combined Chronic Toxicity and Carcinogenicity Study in Rats with 26, 52 and 78 Week Interim Kills (Results after 104 Weeks)." Inveresk Research International, Scotland, Inveresk Project No. 451089. October 30, 1996. Betasan Technical, purity 94.2%, admixed with the feed at concentrations of 0, 1, 15 or 60 mg/kg/day to 30 and 50 rats/sex/group for 78 weeks (toxicity study) and 104 weeks (oncogenicity study), respectively. ChE NOEL = 1 mg/kg/day (Inhibition of plasma and red blood cell cholinesterase). Systemic NOEL = 15 mg/kg/day. At the high dose, decrease of red blood cell parameters and body weight, and an increase in the incidence of hepatocyte vacuolation, eosinophilic foci in the liver and liver weight). No evidence of oncogenicity. ACCEPTABLE. (Kishiyama, J., and Iyer, P. 2/9/98)

CHRONIC TOXICITY, RAT

020 039082, "24-Month Chronic Feeding Study in Rats, Prefar (Betasan) Technical, Final Report", (Frederick E. Reno, Hazleton Laboratories America, Inc., Vienna, VA., Report T-6155, 12 April 1979). 60 Sprague-Dawley albino rats per sex per group received Prefar (bensulide) technical (97.6% purity) at dietary concentrations of 0 (Purina® Laboratory Chow®), 1, 4, and 16 mg/kg/day for 104 weeks. Chronic and Oncogenicity NOEL $\geq$ 16 mg/kg/day. Cholinesterase NOEL = 4 mg/kg/day (> 20% decrease in RBC and plasma ChE activity at 16 mg/kg/day in both sexes compared to controls). **Unacceptable** and not upgradeable (dosing justification not provided, no ophthalmology, incomplete serum chemistry). (H. Green and P. Iyer, 12/31/97).

Subchronic:

****078 150927**: Mulhern, M., P. Hudson and E. Snodgrass. "Bensulide: 13 Week Subchronic Dietary Toxicity Study in Rats." Inveresk Research International, Scotland, IRI Project No. 451068. September 17, 1992. Betasan Technical, purity 92.4% was admixed with the feed at concentrations of 0 (acetone), 5, 15, 45 or 100 mg/kg/day to 10 Sprague-Dawley rats/sex/group for thirteen weeks. **Possible Adverse effect: Plasma (19- 90%), red blood cell 38-66%), and brain cholinesterase (18-58%) inhibition reported in dose groups, 5, 15, 45 and 100 mg/kg/day. Hematological and serum chemistry changes were noted for the 15, 45 and 100 mg/kg/day groups for males. Reduced body weight gain and increased liver weights and hepatic vacuolation were observed for the high dose group**. **NOEL = 5 mg/kg/day nominal** (Kishiyama and Iyer, P. 2/6/98). Acceptable.

CHRONIC TOXICITY, DOG

085, 086, 087, 088, 089, 090 165404, 165405, 165406, 165407, 165408, 165409 "52 Week Oral (Dietary) Chronic Toxicity Study in Dogs", (D. R. Smith and I. Dean, Inveresk Research, Tranent, EH33 2NE, Scotland, Report # 11478, 11 June 1996). 4 Beagle dogs/sex/group received Betasan Technical with 92.4 ± 0.5% purity (bensulide) in the diet at 0, 0.5, 4.0, and 30.0 $mg/kg^{-1}/day^{-1}$ for 52 weeks. Relative liver weights were increased at all treatment levels in both sexes. Mild vacuolation in the liver was increased for high dose females. Group mean female bodyweights at the high dose level were reduced 5% to 14% compared to controls from week 4. Chronic NOEL = 4.0 mg/kg/day. Reductions of 20%, 55%, and 70% were seen for plasma cholinesterase values (group means) in both sexes at the low, mid, and high dose levels respectively relative to controls. Cholinesterase NOEL < 0.5 mg/kg/day. **Adverse effects are not indicated. Acceptable. (H. Green, and P. Iyer, 3/17/99).

086 165405 A two-part study; Part I: Oral administration of Bensulide (92.4%) via the diet to one male and one female beagle dog for 7 day periods at increasing dose levels of 5, 25, 50 and 100 mg/kg/day. A reduction in body weight and food consumption at the highest dosage was noted along with high liver weights. Part II: Oral dosing of one male and one female beagle dog with Bensulide at 50 mg/kg/day for fourteen days resulted in high AST, ALT, AP and LDH values and high liver weights. Authors of the study concluded that 100 mg/kg/day exceeded the MTD and 50 mg/kg/day is at or near the maximum tolerated dose. No worksheet. P. Iyer, 3/17/99.

087 165406 Four groups of one male and one female beagle dog were dosed daily with Bensulide (92.4%) diet for four weeks at 2, 10, 30 or 50 mg/kg/day. An untreated group was maintained as the control group. Reduced body weight gain was noted in the female at 50 mg/kg/day and a reduction in RBC and plasma cholinesterase activity was also noted at 10, 30 or 50 mg/kg/day. Cholinesterase acitivity was reduced in the cerebellum at 50 mg/kg/day and in the pons at 30 (-25%) and 50 (-64%) mg/kg/day. NOEL was considered to be 2 mg/kg/day. No worksheet. P. Iyer, 3/16/99.

088 165407, "13 Week Oral (Dietary) Toxicity Study in Dogs", (I. Dean and F. Jackson, Inveresk Research International, Tranent, EH33 2NE, Scotland, Report # 11053, 15 May 1995). 4 Beagle dogs/sex/group received bensulide (Betasan Technical with 92.4 ± 0.5 % purity) in the diet at 0, 1, 3, 10, or 30 mg/kg/day for 13 weeks. Activated partial thromboplastin time (KCCT) was increased for high dose males and females at 6 and 13 weeks and for females at 10 mg/kg/day at 13 weeks. No clinical signs were reported. Plasma Cholinesterase was reduced at all doses increasing with dose level at 6 and 13 weeks, 37% to 79% for males and 19% to 78% for females compared to controls. RBC cholinesterase was unaffected except at the high dose (-12% in males, -22 % in females). Relative liver weights were increased for males at 10 and 30 mg/kg/day and for females at the high dose. An increase in periportal fat stain positive in livers was noted for high dose animals. Systemic NOEL = 3 mg/kg/day (liver effects, PTT increase). Brain (pons, cerebellum) cholinesterase not inhibitied at 13 weeks in males, but was decreased by 36% in females at 30 mg/kg/day. Study acceptable (No worksheet). P. Iyer, 3/17/99.

089 090 165408, 165409 Validation of analytical methods for the analysis of bensulide in the dog diet over a range of 10 ppm to 2500 ppm. No worksheet. P. Iyer, 3/17/99.

040 075875, "A Twelve Month Oral Toxicity Study in Dogs with Betasan® Technical", (Carol Auletta, Bio/dynamics, Inc., N. J., Study # T-12939, 21 April 1989). 5 Beagle dogs/sex/group received Betasan® Technical (93.8% purity) by gavage (7 days/week) for 12 months at 0 (corn oil), 2, 10, and 50 mg/kg/day. 5 males and 4 females died or were killed moribund between days 54 and 150 with symptoms prior to death including diarrhea, hypothermia, lethargy and emaciation. Reduced 3, 6, and 12 month plasma ChE at 2 and 10 mg/kg/day (50% and 70% reduction respectively). **Adverse effects**: histopathology revealed biliary proliferation/hyperplasia in 4 dogs/sex/group at 50 mg/kg/day and to a lesser degree in 4 females at 10 mg/kg/day. Also, degeneration of the femoral biceps was noted in 1 male and 3 females at 50 mg/kg/day and to lesser degree in 1 female at 10 mg/kg/day. Chronic NOEL = 2 mg/kg/day (biliary hyperplasia and muscle degeneration in females at mid dose). ChE NOEL < 2 mg/kg/day (pons ChE). **Unacceptable**, possibly upgradeable with submission of dose justification and/or the dose-range finding study. (H. Green and P. Iyer, 2/3/98).

ONCOGENICITY, RAT

See under combined rat above.

ONCOGENICITY, MOUSE

**081 150938: Willerton, C. Atkinson and M. Peterson-Jones., "Bensulide: 78 Week Dietary Carcinogenicity Study in Mice with 52 Week Interim Kill." Inveresk Research International, Scotland, Inveresk Project No. 451073. October 30, 1996. Betasan Technical, purity 94.2%,

admixed with the feed at concentrations of 0, 1, 50 or 200 mg/kg/day and fed for a period of 52 and 78 weeks to 10 (satellite group) and 50 (carcinogenicity group) mice/sex/dose, respectively. ChE NOEL = 1 mg/kg/day. Systemic NOEL = 50 mg/kg/day (body weight and liver effects). Adrenal weight was increased 87%, 126% and 191% for low, mid and high-dose males (no histopathology); body weight was reduced (at least 10%); and plasma, red blood cell and brain cholinesterase reduced 88-97%, 32%-50% and 12-14%, respectively, for mid and high dose groups. Increased incidence of atypia, clear cell foci, and hepatocyte and Kupffer cell pigmentation of the liver increased for the high dose group; and red cell parameters reduced 7-8% high dose females and liver weight increased 43% for high dose males. ACCEPTABLE (Kishiyama, J., and Iyer, P. 2/18/98).

080 150929: Mulhern, M., J. Finch., "Bensulide: 13 Week Dietary Dose Range Finding Study in Mice." Inveresk Research International, Scotland, IRI Project No. 451052. 9/17/92. Betasan Technical, purity 92.4%, was admixed with the feed at concentrations of 0 (acetone), 30, 100, 300 or 1000 mg/kg/day to 10 CD-1 mice/sex/group for thirteen weeks. Food consumption was reduced 7-36% for the three highest dose groups; and body weights reduced 15-24% for 300 and 1000 mg/kg/day males and 13% for high dose females. Incidence of prominent lobulation/enlargement and hepatocyte hypertrophy correlates with liver weight increase for the three highest dosed groups. Ovary and spleen weight decreased 43% and 26% for high dose females. No hematology, clinical chemistry or urinanalysis. Limited tissues for histopathology, no ophthalmology. NOEL = 100 mg/kg/day (decreased body weights). Supplemental information. (Kishiyama, J and P. Iyer, 2/17/98).

021 039083, "Lifetime Oral Study in Mice", (International Research and Development Corporation, Mattawan, MI., Report 153-010, 9/5/79), Prefar (Betasan) Tech., 97.6% purity, fed in the diet for 102 weeks at 0 (Purina® Laboratory Chow®), 10, 30, and 100 mg/kg/day with 60 Charles River CD®-1 mice/sex/group (interim sacrifice of 10/sex/dose at 52 weeks). Increased group mean relative (%) liver weights at 12 and 24 months in 100 mg/kg/day males. **No adverse effect** indicated. **Unacceptable**, possibly upgradeable with submission of dose level justification and dosing solution verification (H. Green and P. Iyer, 1/9/98).
Note: this is a more complete version of 015 028395.

REPRODUCTION, RAT

091, 092 165410, 165411, "Two Generation Reproduction Study in Rats", (S.J. Barton and M. Hastings, Inveresk Research International, Scotland, Report # 11430, 23 February 1996). 28 (F0 generation) or 24 (F1 generation) Sprague-Dawley Charles River CD (outbred albino) rats per sex per group received Betasan Technical (92.4%) in the diet at 0, 25, 150, and 900 ppm through 2 generations with 1 litter per generation. Brain cholinesterase activity was 72% lower for F0 females at the high dose compared to controls but not for F0 males; brain cholinesterase was decreased in F1 males (14%) and F1 females (51%). Erythrocytecholinesterase activity was decreased at 900 ppm with variable results at 150 ppm. Parental NOEL = 150 ppm (decreased brain cholinesterase activity and decreased body weight). Reduced F2 pup survival, (not statistically significant) was noted at 900 ppm. Reproductive NOEL = 150 ppm. **Adverse reproductive effects are not indicated. Acceptable. (H. Green, and P. Iyer, 6/18/99).

022 039084, "3 Generation Reproduction Study in Rats", (Edwin I. Goldenthal, International Research and Development Corporation, 11/29/78), Prefar (Betasan) Technical, 97.6% purity, was administered in the diet through 3 generations with 2 litters/generation (100 day pre-mating treatment interval/generation) at concentrations of 0 (Purina® Laboratory Chow®), 1, 4, and 16 mg/kg/day (nominal) with 15 male and 30 female Charles River CD® rats per group. **No adverse reproductive effects** indicated. Systemic NOEL $\geq$ 16 mg/kg/day. Reproductive NOEL $\geq$ 16 mg/kg/day. **Unacceptable**, upgradeable upon submission of dietary analysis. (H. Green and P. Iyer, 7/30/98).
Note: this is a more complete version of 015 028397.

TERATOLOGY, RAT

024 039090, "A Teratology Study in CD® Rats with Betasan®", (J. L. Minor, Stauffer Chemical Co., CT., Report # T-11896, 9 April 1985). 25 or 26 mated female Sprague-Dawley rats per group received Betasan technical (92.8% purity, Lot # EHC-0586-03[a]) administered by gavage on days 6 through 20 of gestation at concentrations of 0 (corn oil), 5.5, 23.0, and 95.0 mg/kg/day (analytical). Maternal effects reported at 95 mg/kg/day included tremors, reduced bodyweight, reduced food intake, plasma ChE inhibition (84% inhibition compared with controls), and increased relative liver weights. **Teratogenicity is not indicated. Maternal NOEL = 23 mg/kg/day (clinical signs, body and organ weights). Plasma ChE NOEL = 5.5 mg/kg/day (50% inhibition at 23 mg/kg/day). No statistically significant inhibition of RBC cholinesterase levels. Teratogenic NOEL $\geq$ 95 mg/kg/day. **Acceptable**. (H. Green and P. Iyer, 8/4/98)
Note: this is a more complete version of 015 028399.

TERATOLOGY, RABBIT

**026 039092, "A Teratology Study in Rabbits with Betasan®, Final Report", (Mark D. Nemec, WIL Research Laboratories, Inc., Ashland, OH., Report # WIL-27025, 10 October 1985). 18 artificially inseminated female New Zealand White rabbits per group received Betasan® Technical (92.9% purity) administered by gavage on gestation days 7 through 19 at 0 (Mazola® corn oil), 5, 20, and 80 mg/kg/day. Reduced mean maternal body weight (9% on gestation day 19), bodyweight gain (gestation days 7-19), and reduced food consumption (gestation days 7-19) were reported at 80 mg/kg/day. Decreased defecation and urination were also recorded at 80 mg/kg/day. Maternal NOEL = 20 mg/kg/day. Teratogenicity is not indicated. Developmental NOEL = 20 mg/kg/day. Acceptable. (H. Green and P. Iyer, 8/6/98).

GENE MUTATION

023 039086, "Prefar® (Betasan® Technical) (Lot No. WRC-4921-32-14), Mutagenicity Evaluation in *Salmonella Typhimurium*", (Jenness B. Majeska, The *In Vitro* Toxicology Section, Environmental Health Center, Stauffer Chemical Co., Farmington, CT., Report # T-11917, 17 September 1984). *Salmonella typhimurium* strains TA-98, TA-100, TA-1535, and TA-1537 were exposed in triplicate to Prefar (Betasan Technical, 92.9% purity), in the presence and absence of activation (Aroclor 1254 (500 mg/kg) induced male Sprague-Dawley rat or B6C3F1 mouse liver S9 fraction), at untreated, 0 (DMSO), 0.005, 0.014, 0.037, 0.041, 0.111, 0.123, 0.333, 0.370, 1.000, 1.111, 3.000, 3.333, 10.000, 25.000 or 50.000 ul/plate for 48 hours. **An increase in the reversion rate is not indicated. **Acceptable**. (H. Green and P. Iyer, 1/15/98).

044 089219, "Bensulide - An Evaluation of Mutagenic Potential using S. Typhimurium", (R. D. Callander, ICI Central Toxicology Laboratory,UK., Report # CTL/P/3197, 25 February 1991). *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to bensulide (92.7% w/w purity) concentrations of untreated, 0 (DMSO), 1.6, 8.0, 40.0, 200.0, 1000.0, and 5000.0 µg/plate for 72 hours with and without rat liver activation in two trials. Treated cultures were exposed in triplicate, vehicle in quintuplicate, and untreated in duplicate. **An increased reversion frequency is not indicated. **Acceptable**. (H. Green and P. Iyer, 1/28/98).

**023 039087, "Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test Forward Mutation Assay", (Jenness B. Majeska, The *In Vitro* Toxicology Section, Environmental Health Center, Stauffer Chemical Co., Farmington, CT., Report # T-11918, 18 September 1984). L5178Y (TK+/-) mouse lymphoma cells were exposed (4 hours) to Prefar/Betasan Technical (92.9% purity), in duplicate, with activation (Aroclor 1254 (500 mg/kg) induced male Sprague-Dawley rat liver S9 fraction) at untreated, 0 (DMSO), 0.040, 0.045, 0.050, 0.055, 0.060, or

0.070 µl/ml and non-activated at untreated, 0, 0.006, 0.008, 0.010, 0.012, and 0.014 µl/ml. **An increase in the frequency of mutations at the thymidine kinase locus is not indicated. Acceptable**. (H. Green and P. Iyer, 1/16/98)

CHROMOSOME EFFECTS

023 039088, "Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test Cytogenetic Assay", (Jenness B. Majeska, The In Vitro Toxicology Section, Environmental Health Center, Stauffer Chemical Co., Farmington, CT. 06032, Report # T-11919, 18 September 1984). L5178Y mouse lymphoma cells were exposed (4 hours), in duplicate, to Prefar/Betasan Technical (92.9% purity) with activation (Aroclor 1254 (500 mg/kg, intraperitoneal injection) induced male Sprague-Dawley rat liver S9 fraction) at untreated, 0 (DMSO), 0.02, 0.03, 0.04, 0.05, 0.06, and 0.07 ul/ml and non-activated at untreated, 0, 0.001, 0.002, 0.004, 0.006, 0.008, 0.010, 0.012, and 0.014 ul/ml. **An increase in the frequency of chromosomal aberrations is not indicated. Acceptable. (H. Green and P. Iyer, 1/16/98).

042 095884, "Bensulide: An Evaluation in the In Vitro Cytogenetic Assay in Human Lymphocytes", (N. James and J. M. Mackay, ICI Central Toxicology Laboratory,UK., Report # CTL/P/3198, 18 December 1990). Male and female human peripheral blood lymphocytes cultured for 48 hours were exposed in duplicate to bensulide (technical, 92.7% w/w purity) concentrations of 0 (DMSO), 5, 40, 60, or 80 µg/ml for 3 hours. Cultures were harvested at 72 hours. **Increased chromosomal damage is not indicated. **Acceptable**. (H. Green and P. Iyer, 1/26/98).

DNA DAMAGE

023 039085, "Mutagenicity Evaluation in Bone Marrow Micronucleus", (Majeska, J.B., In Vitro Toxicology Section, Environmental Health Center, Stauffer Chemical Co., Farmingtion, CT., Report # T-11823, 8/31/84), Prefar (Betasan Technical), 92.9% purity, single dose administered by gavage at 0 (corn oil), 200, 400, 600, or 800 mg/kg with 2 to 5 CD-1 mice/sex/group and sampled at 24, 48, or 72 hours. **Increase in the number of micronuclei is not indicated**. **Unacceptable** (no individual data). (H. Green and P. Iyer, 7/31/98).

042 095885, "Bensulide: An Evaluation in the Mouse Micronucleus Test", (J. M. Mackay, ICI Central Toxicology Laboratory, UK., Report # CTL/P/3173, 27 November 1990). 15 C57BL/6JfCD-1/Alpk mice per sex per group received a single oral dose of bensulide technical (92.7% w/w purity) at concentrations of 0 (corn oil), 250, and 400 mg/kg. 5 per sex per group were sacrificed for bone marrow sampling at 24, 48, and 72 hours. **Increased incidence of micronucleated polychromatic erythrocytes is not indicated. Acceptable. (H. Green and P. Iyer, 1/27/98).

023 039089, "Effects of Prefar®/Betasan® on Human Fibroblast DNA", (Ronald D. Snyder, The *In Vitro* Toxicology Section, Environmental Health Center, Stauffer Chemical Co., Farmington, CT., Report # T-11920, 19 September 1984). 30 minute exposure of human foreskin fibroblasts to Prefar® (Betasan® Technical) (92.8% purity) at 1.0 ul/ml followed by 2 hour incubation for the alkaline sucrose velocity sedimentation assay or in the nick translation assay with no incubation interval or with 1.5 hour incubation in the presence and absence of repair inhibitors ara-C and HU. **An increase in DNA damage or repair** was not indicated. **Unacceptable**, not upgradeable (assayed in the absence of activation only). (H. Green and P. Iyer, 1/20/98)

NEUROTOXICITY

****025 039091**, "Acute Delayed Neurotoxicity Study with Betasan® Technical in Adult Hens", (Dr. G.L. Sprague, Stauffer Chemical Co., Richmond, CA., Report # T-6490, 12 April 1982). 10 or 24 White Leghorn hens (H&N Petite)/group received 2 single unprotected treatments of Betasan Technical (93.7% purity), 3 weeks apart, by gavage at 0 (corn oil), 124, 1240, and 3100 mg/kg. 15/24 hens died at 3100 mg/kg. Walking behavior was impaired days 8-29 at 3100 mg/kg and on days 15-22 at 1240 mg/kg. **Possible adverse effect indicated: Sciatic nerve fiber degeneration after 2 doses given 21 days apart. Acceptable** (H. Green, and P. Iyer, 8/6/98).

SUPPLEMENTAL

082 150939, Validation of Analytical Method N0. 5499 For the Analysis of Bensulide in Rodent Breeder (Rat) and Mouse N0. 3) Diet; The Assessment of Diet Mixing Procedures and the Stability of Bensulide in Such Formulations, (K. Fisher and J. R. Martin. Inveresk Research International, Tranent, EH33 2NE, Scotland., IRI Project No. 354994, Report No. 10293, 19 September, 1994). No worksheet.
Reviewed by P. Iyer, 2/20/98.

083 150940 , Validation of Analytical Method N0. 5494 for the Analysis of Bensulide in Rodent Dietary Formulations, (R.G.R. Fleck and K. Fisher., Inveresk Research International, Tranent, EH33 2NE, Scotland., IRI Project No. 354997, Report No. 9343, 10 October, 1996). No worksheet.
Reviewed by P. Iyer, 2/20/98.